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SOCKEYE EGG SURVIVAL COMPARISON USING
OZONE GENERATION AND ULTRAVIOLET IRRADIATION

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TECHNICAL REPORT

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ABSTRACT

Survival of sockeye salmon (Oncorhynchus nerka) eggs incubated in water treated with ozone or ultraviolet irradiation (UV) was compared. Infection of sockeye salmon eggs and resultant fry with infectious hematopoietic necrosis (IHN) virus can result in major mortalities. Pretreatment of incubation water to inactivate the virus may prevent infection by waterborne viruses. Eggs were incubated in treated water for 9 months. No natural challenge with the virus occurred. Significantly greater mortality occurred in the both the ozone treatment group and the UV group when compared to the controls taken during the same time period. Because of the problems associated with use of ozone, it was concluded that UV is the preferential depuration treatment.

KEY WORDS: sockeye salmon, Oncorhynchus nerka, ozone, ultraviolet irradiation, depuration.

INTRODUCTION

Ozone, a strong oxidizing agent, has been found to be effective in destruction of bacterial and viral fish pathogens (Wedemeyer and Nelson 1977, Wedemeyer et al. 1978; Conrad et al. 1975; Colberg and Lingg 1978). In phosphate-buffered distilled water, infectious hematopoietic necrosis (IHN) virus was inactivated in 30 seconds by a residual of 0.01 mg/L (Wedemeyer et al. 1978). An application of 70 mg/h/L for 10 minutes destroyed the virus in hard or soft lake waters (op. cit.) Ultraviolet irradiation (UV), frequently used as a bactericide, may be a promising means for destruction of smaller fish pathogens (Burrows and Combs 1968). Ultraviolet is a mutagen which distorts DNA (Conn and Stumpf 1972). It has proved effective for destruction of certain protozoans and is safe to use on fish as long as they are not directly exposed (Giese 1967). Infection of fry with Myxosoma cerebralis was prevented by treatment of contaminated water with UV (Hoffman 1974). Both UV and ozone treatment may be valuable in fish disease control as they leave little or no residuals. The half life of ozone is about 15-25 min in aqueous systems but it can exist in the air for long periods (Layton 1972). Coho salmon (Oncorhynchus kisutch) reared in ozone-treated seawater have appeared to be healthier and have less scale loss than those reared in untreated water (Danielson 1975).

The objective of this study was to determine the effectiveness of the two treatments in controlling IHN virus in hatchery reared sockeye (Oncorhynchus nerka) eggs and alevins. When no natural challenge from IHNV was apparent, we analyzed the data collected for possible egg treatment mortality differences. This cooperative research included participation by Big Lake Hatchery personnel, Anchorage Fish Pathology Laboratory (AFPL) and the National Fisheries Research Center (NFRC) of the U.S. Fish and Wildlife Service, Seattle, Washington.

MATERIALS AND METHODS

Treatment Groups

We divided sockeye salmon eggs, from Meadow Creek near Big Lake Hatchery (61°32' N, 149°54' W), into three groups. Two treatment groups were supplied with water treated by ozone and by UV respectively. The third group, the control, was supplied with untreated hatchery water. Each group consisted of five McMullen-Zenger (MZ) incubators, seeded at a density of approximately 100,100 sockeye salmon eggs per box. We disinfected all eggs with an iodophor prior to seeding.

Water

For all three groups, water was sand filtered before use; water flow was 88 gpm. All eggs received formalin at 4 ppm three times a week.

Ozone Generator

We supplied the ozone generator (Model HCZF-18; Pollution Control Industries Stanford, Conn.) with high purity oil-free compressed air at dewpoint of -40°C (air compressor Model PS30; Pollution Control Industries). Ozone generated was bubbled into the water in an aspirator column at the depth of 1.2 M. The ozone level at the top of this column was adjusted to 10 to 50 ppb; the water was exposed to this ozone level for approximately 1 min. After the water was ozonated, it was mechanically agitated with air stones to decrease residual ozone levels to less than 10 ppb at the headbox. Any free ozone escaping from the aspirator column was exhausted out to protect hatchery personnel. The experimental apparatus is shown in Figure 1.

Measurement of Ozone

Air ozone levels may not exceed 100 ppb according to Occupational Safety and Health Association. Ozone measurements were taken with a Hach "Direct Reading" Colorimeter. Periodic ozone measurements assured levels of 10 to 50 ppb at the top of the aspirator column, and levels of 10 ppb at the headbox and in the incubator effluent. Deaeration was established above the headbox by adding more air stones to the water flow in an attempt to reduce any residual ozone levels. The Hach kit did not accurately measure ozone levels less than 10 ppb in water.

Ultraviolet Irradiator

Water purification for the UV treatment group was accomplished by passing all of this group's incoming water through an 18-lamp UV irradiator (Steroline water sterilizer Model PVC 18; Aquafine Corp. Los Angeles). This irradiator can sterilize water flowing under the lamps at a rate of up to 490 lpm, quite adequate for our 330 lpm water flow. Figure 2 illustrates the water sterilizer.

Egg Picking

During the first egg picking operations, one egg picker was used for all groups. Between the picking in each group, the picker was rinsed with water of the upcoming group (i.e. if UV eggs were to be picked, the machine was rinsed with UV treated water beforehand). During egg picking operations, each group of eggs remained in their own treated water.

Incubation

Eggs were incubated with treated water for over 9 mo. Fry were enumerated by weighing methods. Water displacement was utilized for egg enumeration (± 0.7% error).

RESULTS

Survival for Individual Boxes

Egg and emergent fry numbers for each incubation box are summarized in Tables 1 and 2. Survival from egg to emergent fry was greatest in the UV group with 89.0% followed by the controls at 88.2% and the ozone group at 78.6%. The dates of 50% emergence fell within a 7 d span from 28 April 1977 to 4 May 1977. Significantly greater mortality occurred in the box nearest the influent than in the other boxes for all three groups ($p < 0.001$). Mortality in the box nearest the effluent was significantly less only for the two treatment groups ($p < 0.001$). These factors suggest ozone dosage may be the mortality differential factor. We performed statistical analyses using chi-square analysis.

Mortality per Developmental Stage

Eggs treated with ozone until eyeing showed the greatest mortality per developmental stage at 20.4% (Table 3). Conversely, the ozone group experienced the lowest mortality in the eyed egg to emergent fry stage (1.2%). The UV groups showed the least mortality of all test groups in eggs up to eyed stage (4.0%). Percent mortality was slightly greater in UV group (7.3%) than in controls (6.2%) from eyed eggs to emergent fry. Mortality in controls from seeding to eyed egg was significantly less than from eyed egg to emergent fry ($p < 0.001$). Total mortality for all developmental stages was greatest with ozone treatment (21.4%) and least with UV treatment (11.0%). Chi-square analyses (Table 4) revealed that the differences were significant ($p < 0.001$).

Mortalitity per Egg Take Date

Eggs were collected on five different days. Egg mortality data to eyeing was divided into two groups based on egg take date. The early egg take group consisted of eggs taken from 8/15-8/18 and the late group from 8/19-8/22. Chi-square analysis was used to determine if the egg take date affected the mortality (Table 4). For treatment and control groups combined, mortality to eyed egg was significantly higher in the late group ($p < 0.001$). Within the control group, mortality was greater in eggs taken during the later period. All eggs in the ozone treatment group were taken during the later period and all in the UV group were taken in the earlier period. Analysis comparing each of the treatment groups to the control eggs taken during their respective periods revealed that mortality to eyeing was significant in each treatment group with it being more substantially so in the ozone group.

DISCUSSION

Egg Mortality

The significant difference in mortality relative to the egg take date suggests that a variable such as water temperature may have played a part. Higher water temperatures increase the activity of Saprolegnia. The Saprolegnia infestation level was more profuse on the eggs in the ozone group. Initially, it appeared that the mortality related to egg take date may have precipitated significant differences but further analyses revealed that treatment group mortalities were higher regardless of egg take date.

High mortality in eggs treated with ozone until they are eyed suggests exceptional sensitivity to ozone during that period. In addition, ozone concentration may have been excessive during early phases of the experiment. A bioassy run concurrently indicated that the ozone levels were not toxic ($P < 0.01$). The DPD Chlorine (N, N-Diethyl-p-phenyl-enediamine) method modified for ozone has an overall precision of approximately 0.12 ppm (American Public Health Association 1976). This may be too large of a concentration for long-term exposure. Under production conditions using ozone treatment, accurate measurement of 1 ppb would be desirable for hatchery use.

When compared with the control eggs taken during the same period, a slightly higher mortality to eyeing was evident in the UV group. Apparently some sensitivity to UV irradiation is present then as well as during the next stage. The eyed egg to emergent fry is the most critical period for exposure to UV. Mortality during that stage is nearly double that of the previous stage for the UV group. If mortality during this critical period could be reduced, either UV or ozone treatment could be useful in reducing mortality from IHN virus.

Toxicity to Humans

The effects of ozone on humans varies from coughing and sneezing to nausea and death depending on the concentration and exposure. An ozone concentration of 0.1 ppm is considered unsafe for continuous exposure of operating personnel (Layton 1972). People can detect the odor at approximately 0.02 to 0.05 ppm. Irritation of the nose and throat can occur at levels as low as 0.05 ppm. Ultraviolet irradiation leaves no residual and is not toxic but can be damaging to the eyes.

Saprolegnia

Hatchery personnel commented on the profuse growth of Saprolegnia in incubation boxes treated with ozone. Higher Saprolegnia levels in incubators treated with ozone may have been related to higher mortalities there. In contrast, other work suggests that ozone may control fungus on eggs by non-selective oxidation (Benoit 1966).

Usefulness of Ozone and UV

Ozone can be beneficial if the 20% mortality can be reduced in the seeding to eyed egg stage. Perhaps using lower doses initially will reduce these. In addition to being virucidal, ozone is bactericidal especially when combined with sonication (Burleson et al. 1975). It could possibly be utilized in facilities where furunculosis is a problem. Other applications of ozone include treatment of hatchery effluent, oxidation of nitrogen compounds for recirculating systems and decolorization of malachite green1. It may also have considerable application in hatchery or wet lab disinfection of fish and microorganism containers. Ultraviolet irradiation can also be used for sterilization of water but turbidity and organic contents should be taken into consideration in establishing application rates. From this data, we conclude that between UV and ozone, UV is the depuration technique of choice because measuring difficulties, toxic fish effects, and human health problems are associated with ozone use.

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Table 1. Egg and fry numbers during experimentation.

Treatment group	Box	Date eggs collected	Initial egg number	Eggs after first pick off	Emergent fry number	Date of 50% emergence
ozone [a]	5A	8/19/76	93,900	82,937	77,778	4/28/77
	5B	8/20/76	100,100	73,554	71,559	5/2/77
	5C	8/20/76	100,100	76,524	73,453	5/2/77
	5D	8/20/76	100,100	64,421 [b]	70,848 [b]	5/3/77
	5E	8/22/76	100,100	95,861	94,756	5/4/77
Total No.'s			494,300	393,297	388,394	
UV	6K	8/16/76	100,100	96,674	82,016	4/27/77
	6J	8/15/76	100,100	93,812	87,883	4/28/77
	6I	8/15/76	100,100	100,100	90,149	4/27/77
	6H	8/15/76	100,100	96,706	92,783	4/27/77
	6G	8/15/76	100,100	93,289	92,676	4/28/77
Total No.'s			500,500	480,581	445,507	
control	7J	8/19/76	100,100	89,925	85,467	4/30/77
	7I	8/19/76	100,100	89,269	80,255	5/3/77
	7H	8/16/76	100,100	99,637	92,464	4/28/77
	7G	8/16/76	100,100	96,399	91,492	4/27/77
	7F	8/16/76	100,100	95,417	91,801	4/27/77
Total No.'s			500,500	470,647	441,479	

[a] Sequence of water delivery is from the top to the bottom of the column within each group.

[b] weighing or counting error

Table 2. Percent mortality in MZ boxes by developmental intervals.

MZ box within treatment groups	Percent Mortality		
	Seeding to eyed egg	Eyed egg to emergent fry	Seeding to emergent fry
Ozone			
5A [a]	11.7	6.2	17.2
5B	26.5	2.7	28.5
5C	23.6	4.0	26.6
5D	35.6	10.0[b]	29.2
5E	4.2	1.2	5.3
Mean total[c]	20.43	1.3	21.4
UV			
6K	3.4	15.2	18.1
6J	6.3	6.3	12.2
6I	0	9.9	9.9
6H	3.4	4.1	7.3
6G	6.8	0.7	7.4
Mean total	4.0	7.3	11.0
Control			
7J	10.2	5.0	14.6
7I	10.8	10.1	19.8
7H	0.5	7.2	7.6
7G	3.7	4.8	8.6
7F	4.7	3.8	8.3
Mean Total	6.0	6.2	11.8

[a] Sequence of water delivery is from the top to the bottom of the colmun within each group.

[b] Counting or weighing error.

[c] Mean total is the summation of mortality numbers in individual boxes within a treatment group. It is derived from integers in Table 1.

Table 3. Total mortality in treatment and control groups during egg and fry development.

Mortality occurring between seeding and emergent fry

Treatment group	Mortality number	Percent mortality [a]	Emergent fry number	Seeding density
Ozone	105,906	21.4	388,394	494,300
UV	54,993	11.0	445,507	500,500
Control	59,021	11.8	441,479	500,500
Totals	219,920	14.7	1,275,380	1,495,300

Mortality occurring between seeding and the eyed egg stage

Treatment group	Mortality number	Percent mortality	Number of live eyed eggs	Seeding density
Ozone	101,003	20.4	393,297	494,300
UV	19,919	4.0	480,581	500,500
Control 1 [b]	29,853	6.0	470,647	500,500
Totals	150,775	10.1	1,344,525	1,495,300

Mortality occurring between the eyed egg stage to emergent fry

Treatment group	Mortality number	Percent Mortality [c]	Emergent fry number	Number of live eyed eggs
Ozone	4,903	1.3	388,394	393,297
UV	35,074	7.3	445,507	480,581
Control 2	29,168	6.2	441,479	470,647
Totals	69,145	5.1	1,275,380	1,344,525

[a] Percent mortality = mortality/seeding density

[b] Controls are designated 1 and 2 because of slight difference in mortality during different phases of development. Designation is used for statistical analyses.

[c] Percent mortality = mortality/eyed egg

Table 4. Chi-square (χ^2) tests at the 5% significance level for experimental treatment and control groups of eggs and fry. Mortality occurring between seeding and eyed eggs is used for analyses unless otherwise noted.

Group	χ^2	Critical levels (p=0.05)	Degrees of freedom	p	All H_a's accepted
Ozone:Control[a/]	16,685.0	7.682	1	<0.001	Ozone≠Control
UV:Control[a/]	161.0	7.682	1	<0.001	UV≠Control
Early:Late egg take	80,126.4	7.682	1	<0.001	Early≠Late
Early:Late egg take (control group only)	12,196.2	7.682	1	<0.001	Early≠Late
Ozone:Late egg take control	9,723.7	7.682	1	<0.001	Ozone≠Late
UV:Early egg take control	579.2	7.682	1	<0.001	UV≠Early

[a/] Mortality occurring between seeding and emergent fry is used for analysis.

Figure 1. The ozone treatment system. The ozone generator is visible in the upper left corner of photograph (X). MZ boxes are readily visible in foreground. The arrow shows a portion of the headbox.

Figure 2. The ultraviolet water sterilizer in its operating position.